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Improving Pepper Inbreds for Resistance to Pepper Yellow Leaf Curl Thailand Virus (PepYLCTHV) through Challenged Inoculations

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Abstract: Chili peppers (*Capsicum annuum* L.) are an economically important crop worldwide. *Pepper yellow leaf curl Thailand virus* (PepYLCTHV), a Begomovirus causing yellow leaf mosaic disease of chili pepper, has been reported to incur 95% economic loss under epiphytotic conditions. Thirty-one chili genotypes were screened for resistance to PepYLCTHV disease through inoculation using 10–15 viruliferous whiteflies per plant. We purified two resistant lines (PEP6 and PEP12) through four generations of selfing and selection. At 28 days after inoculations, two chili genotypes (PEP6 and PEP12) had low disease severity and percentage of disease incidence (DI) compared to four susceptible checks, viz., Yodsonkeam80, Homsupan, Huareau12, and Pong Charian, which had a disease severity score of 5 with 100% DI. Thirty initial plants of PEP6 showed an average disease severity of 3.64 with 69.33% DI, and PEP12 showed an average disease severity of 3.83 with 77.67% DI. From these populations, we selected nine highly resistant plant of PEP6 and seven plants of PEP12 having a disease severity of 0 through pure-line selection for four selfing generations. The ratio of resistance (R) to susceptibility (S) consequently decreased. In PEP6, the ratio decreased from 1R:2S to 1R:1S, while in PEP12 the ratio decreased from 1R:3S to 1R:1S. These lines have potential for release as resistant lines for improving chili pepper resistance to PepYLCTHV and for developing makers associated with the resistant trait.

Keywords: artificial screening; begomovirus; breeding for resistance; germplasm resistance to PepYLCTHV

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1. Introduction

Chili pepper (*Capsicum* spp.), a member of the Solanaceae family, is a highly valued cash crop. The global chili pepper production in 2022 was 59.0 million tons from an area of 4.47 million hectares. China, Mexico, Indonesia, Türkiye, and India are primary producers along with Thailand [1]. The productivity of chili peppers has been declining due to climate change [2] and the incidence of insects and diseases [3,4]. The major pepper diseases

prevalent in tropical and subtropical regions are anthracnose, fruit rot, bacterial wilt, and pepper leaf curl virus disease.

Pepper leaf curl virus (PepLCV) belongs to the Begomovirus genus. Begomoviruses are transmitted by whitefly (*Bemisia tabaci*) in a persistent, circulative manner. Many begomoviruses in *B. tabaci* can be infective for longer than the latent period, sometimes for the entire life of the insect [5]. This virus is not known to be transmitted through seeds. Two species, *Pepper yellow leaf curl Kanchanaburi virus* (PepYLCKaV) and *Pepper yellow leaf curl Thailand virus* (PepYLCTHV), were diagnosed and characterized from chili pepper growing areas of Thailand [6]. The virus-causing leaf curl disease was first identified in Indonesia in the early 2000s [7]. The yellow leaf curl disease in chili pepper was initially observed and identified in Thailand in 1995 [8]. Chiemsombat et al. [6] reported a significant increase in PepYLCV cases in 2014 and rapidly expanded to *Capsicum chinense* and *C. frutescens* species in Kanchanaburi province, Thailand. Currently, most of the commercial cultivars of *C. annuum* L. widely grown in Thailand are susceptible to PepYLCV. The disease is difficult to control as the vector of the virus (whiteflies) has a wide host range like okra, tomato, eggplant, soybean, angled luffa, cucumber, bottle gourd, and pumpkin [6,9]. Developing PepYLCV-resistant cultivars in chili peppers has been challenging due to the disease's nature, the vector, and the complex infection strategies of viruses [10,11]. Begomovirus-induced studies on various sources of resistance have revealed qualitative and quantitative gene actions for resistance, depending on the virus strain, chili pepper genotype, and their interaction [12].

The resistant sources to begomoviruses reported in *C. annuum* include 9853-123, PSP11-4 [13,14], DLS-Sel-10, WBC-Sel-5, and PBC142 [15]. However, no commercial chili pepper has been reported as resistant or tolerant to the PepYLCTHV disease and none of these genotypes were purely resistant, as inoculated plants of each entry were segregated into resistant and susceptible plants. So far, no immune genotype has been reported against these viruses, but genotypes showing a very high level of resistant and tolerant reactions have been identified and used in commercial pepper breeding. The resistance inheritance has been reported to be complex varying from polygenic (additive and non-additive epistasis) to monogenic recessive and dominant based on host and strain specific types [9,16–18]. Thus, the purification of resistant plants is imperative.

Mass selection has been an efficient method for populations controlled by polygenic genes. Chili peppers are self-pollinating crops and classical methods such as pure line and mass selection have been utilized for germplasm purification, depending on the heritability trait [19,20]. However, there are no reports on the selection of individual plants resistant to PepYLCV. In this communication, we report a comprehensive approach to select resistant genotypes and improve the resistance levels of individual plants against PepYLCTHV using viruliferous whitefly inoculations.

2. Materials and Methods

2.1. Plant Materials

Plant materials for this investigation consisted of four screening cycles. In the 1st cycle, we screened 31 chili pepper genotypes comprising 24 breeding lines sourced from Khon Kaen University (KKU), Thailand, known for their resistance to virus diseases under field conditions. The remaining lines were selected from the World Vegetable Center, Taiwan (improved lines). Pong Charian and 9853-123 were used as susceptible and resistant checks, respectively (Table 1). A randomized complete block design with three replications was used. Ten plants per replication were located in a square plot. The experiment was performed between January and April 2020. For the 2nd cycle, two resistant lines (PSP11-4 and 9853-123) were selected from the first cycle, based on low disease severity means, and the highly resistant plants (score 0). The seeds of the selected plants were screened for resistance to PepYLCV from June to October 2021 (Figure 1). Based on phenotypic screening and PCR-based virus detection, the resistant line PSP11-4 (coded as PEP6) was selected from a highly resistant genotype (R−), while line 9853-123

(coded as PEP12) was selected from a highly resistant genotype (R+). The seeds from the selected plants of the second cycle were continuously challenged and screened for the third and the fourth cycles from January to May 2022, and October 2022 to April 2023, respectively. Screening, selection, and generation advancement from C_1 (30 plants/line), C_2 (60 plants/line), and C_3 (200 plants/line) generations were performed at Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand. Subsequently, screening and selection of C_4 (100 plants/line) were performed at the School of Agricultural of Technology, King's Mongkut Institute of Technology Ladkrabang, Thailand by artificial whitefly transmission. The number of plant screenings for each cycle depends on the extent of seed settings in resistant plants.

Table 1. Chili pepper genotypes are utilized for screening against (PepYLCTHV).

Source	Type	Genotype (Name)
Khon Kaen University (KKU), Thailand	Advance breeding line	KM-P13001-4, PSP11-10-1, PSP11-10, PSP11-11, PSP11-3-1, PSP11-4, PSP11-5, PSP11-7, PSP11-7-1, PSP11-8, PSP11-8-1, ANT1, ANT3, ANT14, ANT17, ANT18, Pong Charian (susceptible check)
	Local cultivar	Keakdum, Jindanil 80, Yodsonkeam 80, Homsupan, Huareau 12, Huareau 7
World Vegetable Center (WorldVeg), Taiwan	Pure-line	9853-123 (resistant check), PP0237-7508, PP0437-7506, PP0437-7504-1, PP0437-7504-2, PP0437-75041, PP9950-5197, PP9955-15

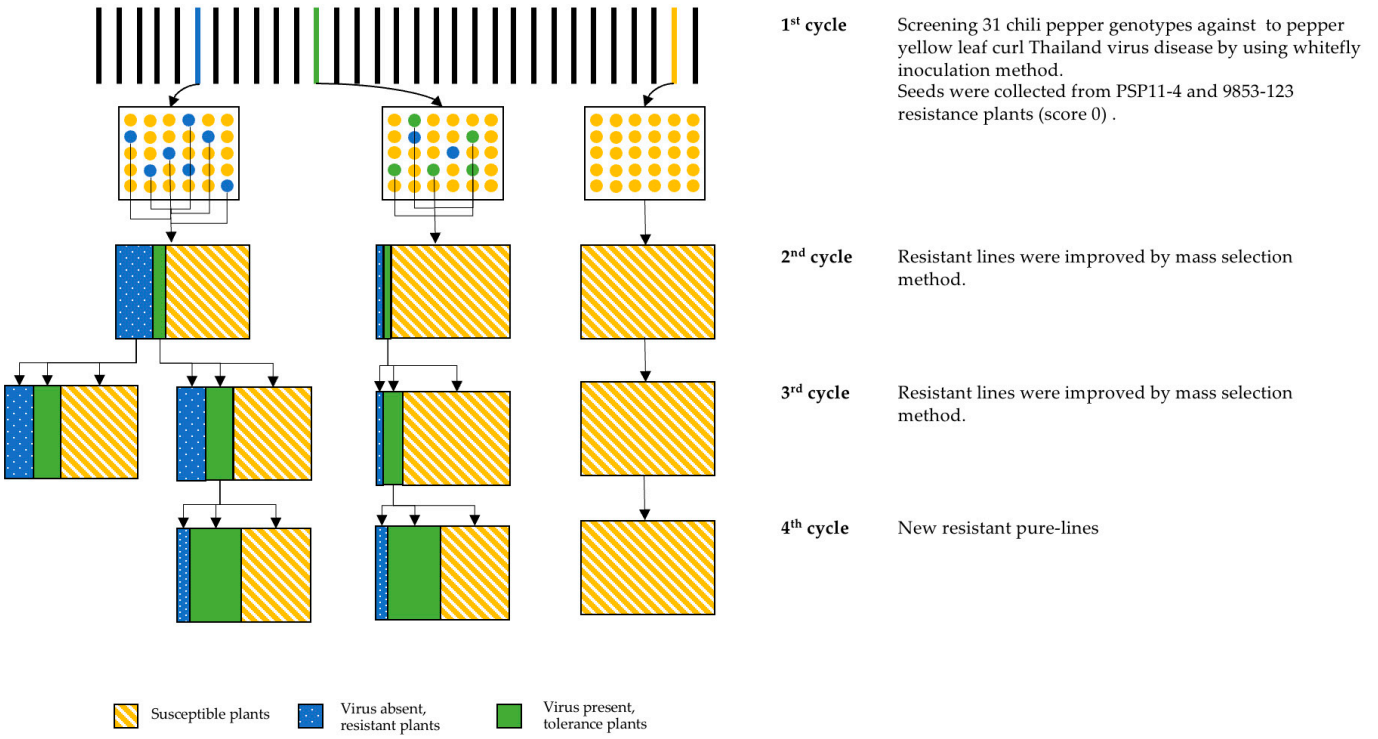

Figure 1. Schematic of screening procedures of chili pepper pure-lines resistant to PepYLCTHV disease.

2.2. Viruliferus Whitefly, Inoculation, and Disease Evaluation

Nonviruliferous whiteflies (*Bemisia tabaci* Genn) were maintained and multiplied on cotton plants (*Gossypium hirsutum*) in insect-proof cages. Adult whiteflies were used for virus transmission and tested purification. The non-viruliferous whiteflies were released on PepYLCTHV-infected chili pepper plants (Pong Charian) for 24 h acquisition access period. Chili pepper leaf and whiteflies were sampled for the confirmation of PepYLCTHV using uni-

versal primers specific for pepper yellow leaf curl, PepYLCTHV-F: 5′ ATGGCGAAGCGTCCCGCAGAT 3′, and PepYLCTHV-R: 5′CTGCAGTTAATTTTGAACCGAACC3′.

Resistance tests were conducted in insect-free greenhouses to keep seedlings free from viruses. Forty-five days after sowing, the seedling was transferred individually to insect-proof net cages 60 mesh size 100 × 100 × 100 cm. A total of 10 to 15 sterile adult whiteflies were introduced to each plant for 48 h (during feeding time, the plants were shanked). After transmission, the whiteflies were euthanized by spraying insecticide (acetamiprid 20% SP).

The disease scores were recorded at 0, 7, 14, 21, and 28 days after inoculation, using 6 score levels (0, 1, 2, 3, 4, and 5) following Suwor et al. [14] (Figure 2, Table 2).

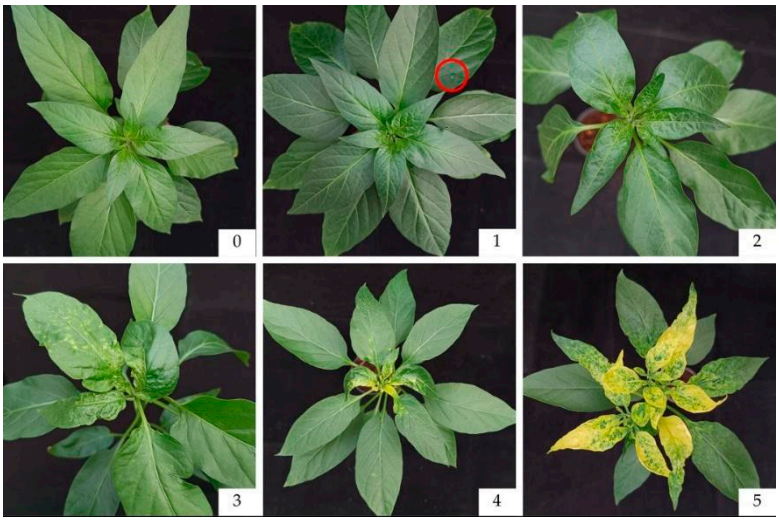

Figure 2. Pepper yellow leaf curl Thailand virus disease severity 6 score levels (0, 1, 2, 3, 4, and 5). The red circle is the 1–2 yellow spots on the mature leaf.

Table 2. Disease scale based on symptom severity.

Score	DI%	Disease Response
0−	No visible symptoms on leaves, virus not detected in PCR test	HR; highly resistant
0+	No visible symptoms on leaves and virus present by PCR detection	T; tolerance
1	0–5% curling and clearing of upper leaves	R; resistant
2	6–25% curling and clearing of leaves, and swelling veins	MR; moderate resistant
3	26–50% curling, puckering, and yellowing of leaves and swelling of veins	MS; moderate susceptible
4	51–75% leaf curling and stunted plant growth and blistering of internodes	S; susceptible
5	More than 75% curling and deformed small leaves, stunted plant growth without flowering	HS; highly susceptible

The 6 score levels were calculated to disease incidence (DI%) using the following formula:

$$DI\% = \frac{\sum(ni \times vi)}{N \times V} \times 100$$

where *i*: 0–5, *ni* = number of symptomatic plants to value of a particular score, *vi* = value symptom score, *N* = the total number of plants observed, and *V* = the highest score value.

Disease severity and percentage disease index were analyzed using variance analysis of variance using the IBM SPSS Statistic version 29.0 software. A chi-square (X^2) test for goodness-of-fit was tested with the ratio of plants resistant to susceptible to PepYLCTHV. The ratio model was considered to be appropriate for a probability (p) value > 0.05.

2.3. DNA Extraction and Verification of Viral Genomes

At 28 DAI, the no-symptom plants of all genotypes were screened for the presence or absence of PepYLCTHV genome using specific PepYLCTHV primers. Total DNA was

extracted from individual plants at 28 DAI using modified CTAB method [21]. Three young leaves were homogenized in 750 µL of 60 °C extraction buffer, which contained 2% CTAB, 10% 1 M Tris HCl (pH 8.0), 28% 5 M NaCl, 4% 0.5 M EDTA, and 0.2% mercaptone. The plant sap was then incubated at 60 °C for 30 min. Next, 750 µL of chloroform:isoamyl alcohol (24:1) was added and mixed. The samples were precipitated by centrifugation at 6000 rpm for 10 min. Afterward, 450 µL of the supernatant was transferred to a 1.5 µL tube. Subsequently, 450 µL of 95% ethanol was added and the mixture was stored at −20 °C for 30 min. The DNA was precipitated by centrifuging at 13,000 rpm for 10 min. The supernatant was poured off, and then 450 µL of 70% ethanol was added and centrifuged for 5 min. The liquid was removed, and the dry DNA was resuspended in 100 µL of dH_2O.

The viral genome of PepYLCTHV was successfully detected using the polymerase chain reaction (PCR) with specific PepYLCTHV primers. The PCR reaction mixture consisted of 1 µL of DNA template, 1 µL of reverse and forward primers, 1 µL of 50 mM $MgCl_2$, 2.5 µL of 10× PCR buffer, 0.1 µL of Taq DNA polymerase, and 18.4 µL of dH_2O. The PCR cycle included an initial denaturation at 94 °C for 1 min, followed by 30 cycles of amplification, with each cycle consisting of 94 °C for 1 s, 55 °C for 2 s, and 72 °C for 2 min. Finally, there was a final extension period at 72 °C for 10 min [22]. The PCR products were then electrophoresed on a 0.8% agarose gel and stained with ethidium bromide for visualization.

3. Results

3.1. Resistance of Chili Germplasms to PepYLCTHV

A total of 930 individual plants of 31 chili pepper genotypes were screened against *Pepper yellow leaf curl Thailand virus* (PepYLCTHV). Upon free-choice whitefly inoculation, all genotypes exhibited disease symptoms with scores ranging from 0 to 5. Disease severity progress was evaluated at 14, 21, and 28 days after inoculation (DAI), with the first symptom appearing at 14 DAI. At 21 DAI, the susceptible check (PEP31) and genotypes PEP15, PEP23, and PEP25 displayed the highest average disease score of 5, while others ranged from 2.90 to 4.96. On the final scoring date, among advanced breeding lines (PEP1, PEP2, PEP3, PEP4, PEP5, PEP6, PEP7, PEP8, PEP9, PEP10, PEP11, PEP26, PEP27, PEP28, PEP29, and PEP30), disease scores ranged from 3.46 to 5.0 and disease indices ranged from 69.33% to 100%. Pure-line chili pepper genotypes sourced from the World Vegetable Center (WorldVeg.) (PEP12, PEP13, PEP14, PEP15, PEP16, PEP17, PEP18, and PEP19) had disease scores ranging from 3.83 to 5.0, and disease indices from 76.67% to 100%. Thailand's commercial chili genotypes (PEP20, PEP21, PEP22, PEP23, PEP24, and PEP25) showed the highest disease scores ranging from 4.96 to 5.0 with disease indices ranging from 99.33% to 100%. Notably, two chili genotypes, PEP6 (advance breeding line) and PEP12 (WorldVeg.), exhibited the lowest average disease scores of 3.46 and 3.83, respectively, with disease indices of 69.33% and 76.67% (Table 3).

Table 3. Average severity of Pepper yellow leaf curl Thailand virus disease (PepYLCThV) in 31 chili peppers challenged by viruliferous whitefly.

Code	Pedigree Name	Disease Score			DI%
		14 DAI [1/]	21 DAI	28 DAI	
PEP1	KM-P13001-4	2.23 $^{f–k}$	4.70 $^{a–e}$	5.00 ^{a}	100.00 ^{a}
PEP2	PSP11-10-1	2.70 $^{c–g}$	4.83 $^{a–d}$	4.83 ^{a}	96.67 ^{ab}
PEP3	PSP11-10	2.20 $^{g–k}$	4.47 $^{a–g}$	4.50 ^{ab}	90.00 $^{a–c}$
PEP4	PSP11-11	2.50 $^{d–j}$	4.67 $^{a–f}$	5.00 ^{a}	100.00 ^{a}
PEP5	PSP11-3-1	2.07 $^{i–k}$	4.20 $^{b–g}$	4.66 ^{ab}	93.33 $^{a–c}$
PEP6	PSP11-4	1.43 ^{l}	3.13 ^{h}	3.46 ^{d}	69.33 ^{e}
PEP7	PSP11-5	1.90 ^{k}	3.90 ^{g}	4.30 $^{a–c}$	86.00 $^{a–d}$
PEP8	PSP11-7	2.13 $^{h–k}$	4.47 $^{a–g}$	4.63 ^{ab}	92.67 $^{a–c}$
PEP9	PSP11-7-1	2.60 $^{d–h}$	4.80 $^{a–e}$	4.83 ^{a}	96.67 $^{a–c}$

Table 3. *Cont.*

Code	Pedigree Name	Disease Score			DI%
		14 DAI [1/]	21 DAI	28 DAI	
PEP10	PSP11-8	2.00 $^{j–k}$	4.27 $^{c–g}$	4.30 $^{a–c}$	86.67 $^{b–d}$
PEP11	PSP11-8-1	2.20 $^{g–k}$	4.03 ^{fg}	4.80 ^{a}	96.00 ^{ab}
PEP12	9853-123	1.17 ^{l}	2.97 ^{h}	3.83 ^{cd}	76.67 ^{de}
PEP13	PP0237-7508	2.63 $^{c–h}$	4.50 $^{a–g}$	4.63 ^{ab}	92.67 $^{a–c}$
PEP14	PP0437-7506	2.33 $^{e–k}$	3.90 ^{g}	4.50 ^{ab}	90.00 $^{a–c}$
PEP15	PP0437-7504-1	2.30 $^{e–k}$	4.90 $^{a–c}$	4.93 ^{a}	98.67 ^{a}
PEP16	PP0437-7504-2	2.50 $^{d–j}$	5.00 ^{a}	5.00 ^{a}	100.00 ^{a}
PEP17	PP0437-75041	2.23 $^{f–k}$	4.67 $^{a–f}$	4.86 ^{a}	97.33 ^{a}
PEP18	PP9950-5197	2.70 $^{c–g}$	4.17 $^{d–g}$	5.00 ^{a}	100.00 ^{a}
PEP19	PP9955-15	2.67 $^{c–g}$	4.40 $^{a–g}$	4.83 ^{a}	96.67 ^{ab}
PEP20	Keakdum	3.00 $^{b–d}$	4.70 $^{a–e}$	5.00 ^{a}	100.00 ^{a}
PEP21	Jindanil 80	2.87 $^{b–d}$	4.80 $^{a–e}$	4.96 ^{a}	99.33 ^{a}
PEP22	Yodsonkeam 80	2.73 $^{c–f}$	5.00 ^{a}	5.00 ^{a}	100.00 ^{a}
PEP23	Homsupan	3.33 $^{b–d}$	5.00 ^{a}	5.00 ^{a}	100.00 ^{a}
PEP24	Huareau 12	2.90 $^{b–d}$	4.77 $^{a–e}$	5.00 ^{a}	100.00 ^{a}
PEP25	Huareau 7	3.33 $^{b–d}$	5.00 ^{a}	5.00 ^{a}	100.00 ^{a}
PEP26	ANT 1	2.53 $^{d–i}$	4.77 $^{a–e}$	5.00 ^{a}	100.00 ^{a}
PEP27	ANT 3	3.13 ^{bc}	4.97 ^{ab}	5.00 ^{a}	100.00 ^{a}
PEP28	ANT 14	2.67 $^{c–g}$	4.85 $^{a–c}$	5.00 ^{a}	100.00 ^{a}
PEP29	ANT 17	2.77 $^{c–e}$	3.97 ^{g}	4.06 $^{b–d}$	81.33 $^{c–e}$
PEP30	ANT 18	2.89 $^{b–d}$	4.15 $^{e–g}$	4.48 ^{ab}	89.23 $^{a–c}$
PEP31	Pong Charian	3.80 ^{a}	5.00 ^{a}	5.00 ^{a}	100.00 ^{a}
CV. (%)		37.7	25.10	22.7	23.0
F-test		**	**	**	**

[1/] Days after inoculation. ** Values in each column followed by difference letters (a–l) indicate they are significantly different at $p < 0.01$. Duncan's Multiple Range Test (DMRT) was the method of mean separation used.

3.2. Number of Resistance Plants and Virus Detection

The response of 31 genotypes to PepYLCTHV at the first screening cycle was assessed across six score levels. Thirteen genotypes displayed symptoms (score 1–5) and no symptoms (score 0). PCR analysis revealed that all inoculated plants were infected with the virus (Figure 3). Plants with a disease score of 0 were further categorized into two groups: those with virus detection (0+) and those without (0−), while scores 1–5 were identified as susceptible to virus detection (Table 4). Among the genotypes, PEP6 and PEP12 had more resistant plants (no symptoms) than others. Specifically, PEP6 had 9 plants classified as 0+ and five plants of PEP12 were classified as 0+ and 2 as 0−. Additionally, various plants from different genotypes, such as PEP2, PEP3, PEP5, PEP7, PEP8, PEP9, PEP10, PEP11, PEP12, PEP13, PEP14, and PEP19, displayed varying levels of 0+ (ranging from one to four plants). The genotypes PEP1, PEP4, PEP16, PEP18, PEP20, PEP21, PEP22, PEP23, PEP24, PEP25, PEP26, PEP27, PEP28, and PEP31 were found to be entirely susceptible to PepYLCTHV.

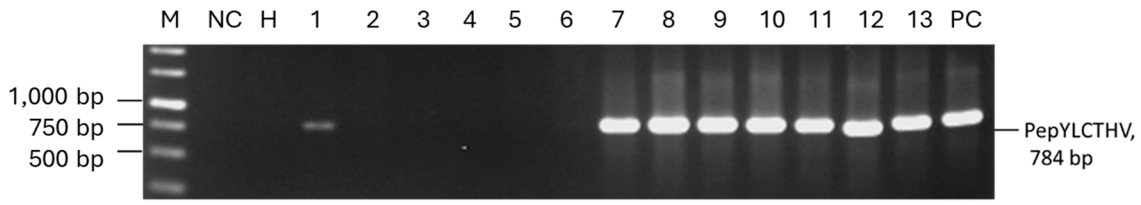

Figure 3. PCR products size 784 bp by specific primer to PepYLCTHV separated by agarose gel electrophoresis: M = 1 kb DNA ladder (Thermo Scientific, Lithuania), NC = negative control (H_2O), H = healthy pepper, PC = positive control (PepYLCTHV, 784 bp), no. 1, 7–13 PCR viral detection of inoculation plants, no. 2–6 no PCR viral detection of inoculation plants.

Table 4. The number of segregated plants to PepYLCTHV disease of 31 chili genotypes at 28 days after inoculation and virus detection using PCR analysis.

Code Number	Number of Scoring Segregation of Inoculated Chili Seedling [1/]							Number of Disease Response [2/]	
	0+	0−	1	2	3	4	5	R	S
PEP1	0	0	0	0	0	0	30	0	30
PEP2	0	1	0	0	0	0	29	1	29
PEP3	3	0	0	0	0	0	27	3	27
PEP4	0	0	0	0	0	0	30	0	30
PEP5	2	0	0	0	0	0	28	2	28
PEP6	0	9	0	0	0	1	20	9	21
PEP7	2	2	0	0	0	1	25	4	26
PEP8	2	0	0	0	0	1	27	2	28
PEP9	1	0	0	0	0	0	29	1	29
PEP10	4	0	0	0	0	0	26	4	26
PEP11	1	0	0	0	0	1	28	1	29
PEP12	5	2	0	0	0	0	23	7	23
PEP13	0	2	0	0	0	1	27	2	28
PEP14	3	0	0	0	0	0	27	3	27
PEP15	0	0	0	0	1	0	29	0	30
PEP16	0	0	0	0	0	0	6	0	6
PEP17	0	0	1	0	0	0	29	0	30
PEP18	0	0	0	0	0	0	30	0	30
PEP19	0	1	0	0	0	0	29	1	29
PEP20	0	0	0	0	0	0	30	0	30
PEP21	0	0	0	0	0	1	29	0	30
PEP22	0	0	0	0	0	0	30	0	30
PEP23	0	0	0	0	0	0	30	0	30
PEP24	0	0	0	0	0	0	30	0	30
PEP25	0	0	0	0	0	0	30	0	30
PEP26	0	0	0	0	0	0	30	0	30
PEP27	0	0	0	0	0	0	30	0	30
PEP28	0	0	0	0	0	0	27	0	27
PEP29	0	0	0	0	14	0	16	0	30
PEP30	0	0	0	0	7	0	19	0	26
PEP31	0	0	0	0	0	0	30	0	30

[1/] 0+ means tolerant plant (score 0 with virus detection), 0− means resistant plant (score 0 with no virus detection). [2/] R means no symptom plant (disease score 0+ and 0−), S means susceptible plant (score 1–5).

3.3. Effective Selection of Resistance to PepYLCTHV

The response to selection for resistance to PepYLCTHV in PEP6 and PEP12 through four advanced cycles is described in Figure 4. The percentage of resistance to the disease in both genotypes showed a slight increase from the first to the fourth cycle (Figure 5). Resistance in PEP6 increased from 30% to 42.78%, and resistance (R) to susceptible (S) ratio 1:2 to 1:1 respectively, and in PEP12 from 23.34% to 40%, R:S ratio 1:3 to 1:1, while susceptibility remained at 100% for the susceptible plants (Table 5). In the initial screening cycle, PEP6 exhibits a disease response score of 0− refer to R−, whereas PEP12 displaying 0+ and 0− refer to R+ and R−, respectively. Plants with the resistant phenotypes R− from PEP6 and R+ from PEP12 were subsequently selfed to assess the disease response within the R− and R+ groups. The resistance of PEP6 (R−) and PEP12 (R+) in the third and fourth exhibited similar segregated responses across three groups: R−, R+, and S. In addition, the morphological characterization of selected chili pepper lines resistant to PepYLCTHV PEP6 and PEP12 was described (Figure 6). They were *C. annuum* L. and showed differences in plant growth, leaf shape, leaf color, flower color, fruit shape, and fruit color. PEP6 was observed to have a green color in plant stem, leaf, and immature fruit, while PEP12 showed a purple color.

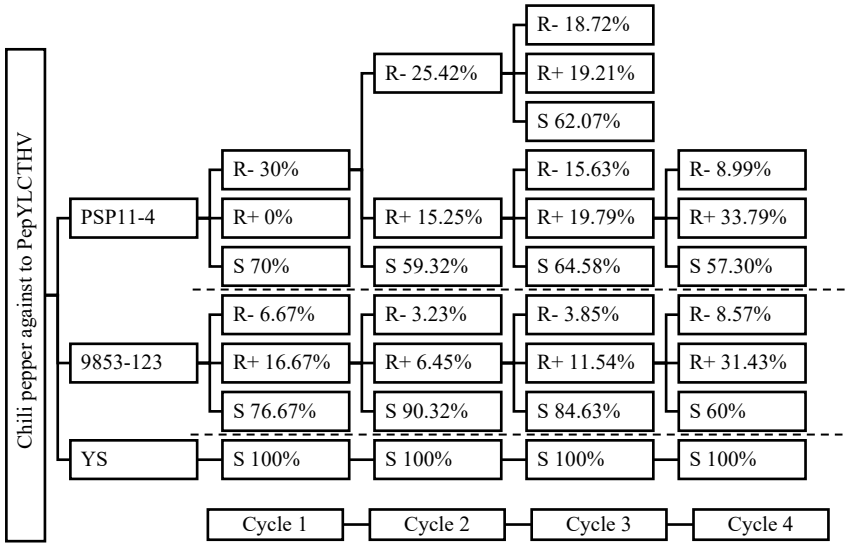

Figure 4. Investigating the resistance levels of PepYLCTHV disease in segregated cycles: R+ is highly resistant with virus presence, R− is highly resistant with the virus absent, and S is susceptible with virus present (through PCR detection method).

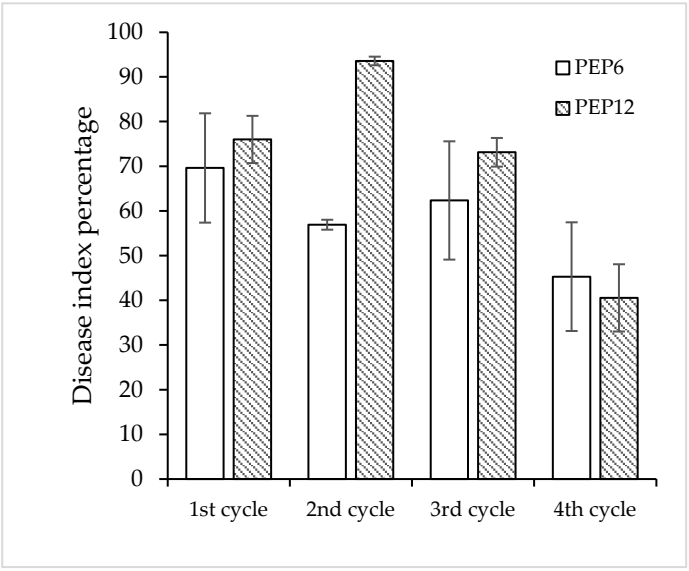

Figure 5. The disease index percentage in four selection cycles of PEP6 and PEP12 at 28 days after inoculation using whitefly inoculation method.

Table 5. Estimates of chi-square (X^2) test for goodness-of-fit of plants resistant to susceptible to PepYLCTHV of PEP6, PEP12, and Yodsonkeam 80 in 4 cycles of selection.

Variety	Cycle	Number of Plants		Best Fit Ratio (R:S Ratio)	X^2	*p*-Value
		Resistance	Susceptible			
PEP6	1	9	21	1:2	0.15	0.7
	2	24	35	1:2	1.43	0.23
	3	111	188	1:2	1.93	0.16
	4	39	50	1:1	1.36	0.24
PEP12	1	7	23	1:3	0.04	0.83
	2	6	56	1:15	1.24	0.26
	3	4	22	1:3	1.28	0.26
	4	15	20	1:1	0.71	0.4

Table 5. *Cont.*

Variety	Cycle	Number of Plants		Best Fit Ratio (R:S Ratio)	X^2	*p*-Value
		Resistance	Susceptible			
Yodsonkeam 80	1	0	30	0:all	-	-
	2	0	30	0:all	-	-
	3	0	30	0:all	-	-
	4	0	30	0:all	-	-

(**a**) (**b**)

Figure 6. Characteristics of virus-free chili peppers against PepYLCTHV include (**a**) characteristics of PEP6 and (**b**) characteristics of PEP12.

4. Discussion

We discovered that two out of thirty-one lines exhibited the highest resistance to PepYLCTHV when subjected to viruliferous whitefly inoculation. One of these resistant lines, PEP6, originated from the World Vegetable Center, Taiwan, was found to be highly resistant under field screenings at Khon Kaen University (KKU) and moderately resistant after graft screenings [14]. This accession is highly resistant for the first time after whitefly artificial inoculation. Additionally, we identified PEP12 (an advanced generation derived from the 9853-123) as resistant to PepYLCTHV as both the absence (immune) and presence (tolerant) of the virus were observed in symptomless plants. PEP12 also demonstrated PepYLCV resistance in field net-house screenings by viruliferous whiteflies at 120 days after inoculation (DAI) and was highly resistant in graft screenings [14]. We observed differences in the responses of individual resistant plants, categorized as R− (virus absent or immune) and R+ (virus present or tolerant). The R− resistance could be attributed to mechanisms such as non-preference by whiteflies, including pre-existing mechanisms like wax, hair, leaf color, and trichomes which initially protect the plant [23,24]. For example, chili genotypes with a high density of glandular trichomes were associated with the whitefly non-preference [25]. The R− resistance may involve the elimination of pathogens from plant cells, known as hypersensitivity. This mechanism entails the production of thickened cell walls, increased cuticle layers, higher trichome density, and the accumulation of secondary products in plants, which collectively deter pests or inhibit pathogen growth, potentially leading to unsuccessful infection expansion [26,27]. In the case of R+ responses, plants may exhibit a local reaction involving the release of molecules triggered by the virus (elicitor), or they may interact with specific proteins produced by both the virus and the plants, such as the nuclear shuttle protein (NSP) and movement protein (MP) These responses can be suppressed by specific proteins or secondary metabolites from resistant host plants, resulting in the restriction or delay of infection [28].

We demonstrated mass selection is efficient in enhancing resistance to PepYLCTHV in two inbred lines. By the fourth cycle of selection, the resistance level increased to

a maximum ratio of 1:1 of resistance to susceptibility, while the agronomic traits and phenotypic appearance were uniform. This evidence suggests that only resistance to PepYLCTHV segregates, possibly indicating that the resistant gene is controlled by more than two genes [17] with one position showing resistance in heterozygous form, likely due to the presence of a lethal gene. The evidence presented elucidated the role of the chlorophyll formation gene in rice leaf pigments, where distinct green (heterozygous) and yellow (homozygous) colors were segregated at a 1:1 ratio among self-progenies from S2 to S6 [29]. In this study, PEP6 and PEP12 were identified as potentially improved sources of PepYLCTHV, which could be used to develop commercial cultivars resistant to PepYLCTHV disease. Future research is underway to understand the genetic mechanisms underlying resistance to PepYLCTHV and enhance our understanding.

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